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GENETICAL STUDIES ON ŒNOTHERA. III

FURTHER HYBRIDS OF *Œnothera biennis* AND *O. grandiflora* THAT RESEMBLE *O. Lamarckiana*[1]

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THE following paper will describe my cultures of *Œnothera,* grown during the season of 1911, which bear upon the working hypothesis announced in a previous contribution (Davis, '11) to the effect that *Œnothera Lamarckiana* arose as a hybrid between types of *O. biennis* and *O. grandiflora.* The cultures of 1911 gave results much more striking than those of 1910 and 1909 and marked progress has been made towards the synthesis of a hybrid between these two species which will be so similar to *Lamarckiana* as to be practically indistinguishable by the usual taxonomic tests.

The problem before me seems to be chiefly that of finding and selecting among the various strains or races of *biennis* and *grandiflora* the most favorable types with which to work. As the study has progressed I have been surprised at the wealth and variety of forms which taxonomically are included in the species *O. biennis,* but which can be readily differentiated in "pure line" cul-

[1] An abstract of this paper was presented before the American Society of Naturalists on December 28, 1911.

tures as biotypes and which so far have held their characters through two and three generations. *Œnothera biennis* is much more rich in biotypes than *O. grandiflora* probably because it is more hardy and adaptive in its life habits, growing over an immense geographical range and under a great variety of climatic and soil conditions. It is clear that any one who cared to give an extended period to the study of *O. biennis* could differentiate scores of elementary species in the assemblage of forms included under this name.

Further acquaintance with *O. Lamarckiana* has led me to believe that under this name must be included a number of races. Excluding the more striking of the "mutants" of De Vries, there still remain strains that differ from one another in such characters as the size of buds and flowers, relative height of the stigma, forms of the capsules, tint of the foliage, etc., and these strains, when selected and carried on in "pure lines" hold their peculiarities and are true biotypes, although the differences between them may be so small as to have little or no taxonomic value. We have then under *O. Lamarckiana* a diverse assemblage and no one will ever be able to prove that any one type is identical with the original since the account of the original and the herbarium material available do not give the information necessary for a full description.

The types called *Lamarckiana,* as now cultivated, have come down to us perhaps entirely from cultures of Messrs. Carter and Company of London at about 1860, but they have come down through diverse channels with abundant opportunities for hybridization and that differentiation that results from selection, conscious or otherwise, on the part of gardeners. In the experimental gardens we are working chiefly with the strains that have passed through the hands of Professor De Vries, who, however, began his work (about 1886) many years after the plant had been in cultivation. This material of the experimental gardens has then been subjected to a large

amount of selection and every worker who is following genetical methods is continuing to differentiate, more or less perfectly, strains or biotypes. I have in my own cultures separated in "pure lines" several strains which differ in the size of the flower, the height of the stigma relative to the tips of the anthers, and the depth of coloration in the foliage, and these strains have held true in my limited cultures.

These points must be borne in mind in judging the results of my studies, for it is one thing to attempt the synthesis of a hybrid *taxonomically* similar to *Lamarckiana,* and it would be quite another to attempt to match exactly a particular biotype of this plant. The probabilities of obtaining a hybrid the exact counterpart of a specific biotype are small; for the reason that very many characters or groups of characters give to these strains their peculiarities. The probabilities of obtaining a hybrid the characters of which will be matched largely or wholly by forms of *Lamarckiana* are, however, in the writer's opinion, excellent and such a type is meant when we speak of a hybrid *taxonomically* similar to *Lamarckiana* or indistinguishable from it.

The announcement of Gates ('10) that certain marginal notes in a copy of Bauhin's "Pinax," 1623, give an accurate description of *Lamarckiana* and establish its presence in Europe at this early date has proved to be a false alarm. These notes consist of matter, copied on the margin of a page, from the longer description of *Lysimachia lutea corniculata* found in the appendix (pp. 520, 521) of the "Pinax." The readiness with which Gates and Hill ('11) have accepted as reliable the statement of Bauhin that the flowers of this form are 3 inches long above the ovary, in the face of the statement a few lines below that the capsules become 2–3 inches long (a manifest absurdity), is inexplicable to the writer. It shows a naïve confidence in the descriptions of the early botanists which is scarcely to be expected in the consideration of so difficult a problem as the origin of *O. Lamarckiana.*

Gates ('11*a*, p. 101) also believes that the description of *Lysimachia Americana* by Hernandez ("Nova Plant. Anim. et Miner. Mex.," p. 882, 1651) is that of *Lamarckiana* "in the strict sense." This account is quite as vague in character as others of the period and the figure is very crude. Gates emphasizes the statement concerning the leaves "sinibus levibus excavata" and regards this as descriptive of the characteristic crinkling of the leaves of *Lamarckiana*. He gives no consideration to the petals clearly drawn as mucronate or to the stigma figured on about the level of the anthers, and is not impressed with the description and figures of the leaves as like the willow. The writer must express his astonishment that an identification of this plant with *Lamarckiana* should be claimed chiefly on a single character loosely described, ignoring important points that radically disagree, and giving no weight to the evident inaccuracy of the description and figure. Gates has changed his position respecting the account of Bauhin which in this later paper (Gates, '11*a*) is referred to *biennis* in agreement with the general opinion of botanists, but I have found no further reference by him to the description of Hernandez.

There have been then no new developments to modify my view that *O. Lamarckiana* was unknown previous to the description of Lamarck's plant at Paris in 1797, about eighteen years after the introduction of *O. grandiflora* at Kew in 1778. There is less reason to lay stress upon the appearance of this plant in the gardens at Paris since the evidence seems clear that the *Lamarckiana* of to-day has genetic relation to the cultures of Carter and Company of London about 1860. I shall have more to say on this point in connection with the valuable sheet in the Gray Herbarium and the interesting history of its probable relation to these same cultures of Carter and Company. This seems to the writer perhaps the most important herbarium sheet known bearing on the problem of the origin of *Œnothera Lamarckiana*. It will be considered in the latter part of this paper.

I have recently had the opportunity of examining two herbarium sheets of American wild plants, referred to in my earlier paper (Davis, '11, p. 227), which were thought by De Vries ('05, p. 386) to be *O. Lamarckiana*. The first of these, at the New York Botanical Garden, is a specimen collected by A. W. Chapman in Florida (1860 or earlier). Duplicates of this material are said to be at the Biltmore Herbarium and at the Missouri Botanical Garden (MacDougal, '05, p. 6). The second sheet, at the Philadelphia Academy of Natural Sciences with an apparent duplicate at the New York Botanical Garden, is of a specimen collected by C. W. Short near Lexington, Ky. This specimen was later considered by Miss Vail to be *O. grandiflora* and a possible escape from cultivation. There is nothing on these sheets from Florida and Kentucky that is not represented in a fair range of herbarium material of *grandiflora* such as may now be found in my own collections and at the New York Botanical Garden. In no point do the sheets closely approach *Lamarckiana* except that they have large flowers. In justice to Professor De Vries it should be stated that he expressed his opinion before the rediscovery of the habitat of *Œnothera grandiflora* by Tracy in August, 1904, and consequently before there was available the extensive material of this species now assembled. Could he have made the comparisons at present possible he would not, I am sure, have given the opinion quoted above.

There has then so far been found in the American herbaria and records, and these have been very thoroughly examined by various workers, no evidence that *Œnothera Lamarckiana* is at present or ever has been a component of the American flora as a wild native species. There are in the south and west certain large-flowered species of *Œnothera* of considerable interest because of their possible affinities with *grandiflora,* and these should be studied by those in a position to do so. A number of these are represented in American herbaria; others in the British Museum are referred to by Gates ('11*a*, p.

591). That an identification with *Lamarckiana* can ever be made from the average herbarium sheet seems to the writer almost impossible, for the specimens of *Œnothera* formerly collected rarely give a fourth of the information necessary to make a critical comparison with *Lamarckiana.* With the clear evidence that the present-day *Lamarckiana* holds a genetic relation to the cultures of Carter and Company, about 1860, the problem of its origin has become much more tangible than formerly and this matter will be taken up in our discussion of these cultures in relation to the sheet in the Gray Herbarium at Harvard University.

American botanists are not likely to believe that *Lamarckiana,* if present in America in 1860, has become so quickly extinct, knowing as they do the vitality of our rich *Œnothera* flora. For example, *O. grandiflora* has actually persisted in the same locality since its first discovery by William Bartram in 1776. Let those interested in the problem of the status of *Lamarckiana* use their best endeavors to discover this plant in the field, but let them give us their results not only by herbarium material covering the entire life history, but above all through seed that can be sent to the workers in the experimental gardens.

The material of this paper will be arranged under the following headings: (1) Methods, (2) Large- and Small-flowered Biotypes of *Œnothera Lamarckiana,* (3) Further Races of *Œnothera biennis* L., (4) Further Races of *Œnothera grandiflora* Ait., (5) Hybrids in the F_1 Generation from the Cultures of 1911, (6) Hybrids in the F_2 Generation from the F_1 Hybrid Plants 10.30*La,* and 10.30 *Lb*, (7) The Probable Composition of the Cultures of Carter and Company from Evidence Furnished by the Sheet in the Gray Herbarium, (8) Further Considerations on the Possible Origin of *Œnothera Lamarckiana* as a Hybrid of *O. biennis* and *O. grandiflora.*

As in previous seasons, I am greatly indebted to the Bussey Institution and to the Botanic Garden of Harvard

University for the facilities that have made possible these studies.

1. Methods

The methods of culture were the same as those of the previous season (Davis, '11, p. 196). I have, however, adopted the system of collecting and sowing seed capsule by capsule as being the safest way of regulating the size of the cultures and obtaining a fair average of results both qualitative and quantitative. Furthermore the seeds which go into a seed pan are counted so there is obtained some data on the percentage of germinations. The count can not be made strictly accurate, for there are in *Œnothera* varying proportions of obviously abortive seeds which so grade into seed of questionable fertility that good and bad could not be separated unless dissected. Nevertheless, these counts are important, especially in cases where it is fundamental that all fertile seed be germinated, as in the comparison of reciprocal crosses.

2. Large- and Small-flowered Biotypes of *Œnothera Lamarckiana*

The experience of the writer during the past six years has forced upon his attention the fact that there is a wide range in the bud and flower measurements of *Œnothera Lamarckiana* in cultures that are practically indistinguishable as to their vegetative characters.

De Vries in his "analytical table of flowers, fruits and seeds" ("The Mutation Theory," Vol. I, p. 452, 1909) gives the measurements of the petals of *Lamarckiana,* on the average, as 3–4 cm. long. I am growing strains or biotypes of *Lamarckiana* derived from seeds of De Vries in which the petals measure from 4–4.5 cm. in length and similar strains have been sent to me from England, where essentially the same form is cultivated under the name *biennis* var. *grandiflora.* These very beautiful plants constitute a sort of élite race and apparently represent

the best that the gardener's art (probably in large part selection more or less in "pure lines") has been able to accomplish.

There have, however, twice come to me seed of *Lamarckiana* through different sources (all originally from De Vries) that has given numbers of plants with much smaller flowers, but otherwise presenting essentially the same characters as the large-flowered types. From such plants I have had no difficulty in establishing strains (*B, D, Y,* and *Z*) in which the petals measure about 2.5 cm. (for figure, see Davis, '11, p. 216). The strains have been perfectly true through two generations, although the cultures have been small. The stigmas in these plants are about on the level of the tips of the anthers, sometimes a little above, in one strain (*B*) somewhat below. In this respect the flowers resemble those of *biennis* in sharp contrast to some of the large-flowered *Lamarckiana* in which the stigma is 6–7 mm. above the tips of the anthers, even higher than is typical of *grandiflora*. The point should be emphasized that when these small-flowered plants are grown side by side with the large-flowered forms there is no hint of important differences between the plants until the time of flowering, when the large and small buds first clearly define the two types.

Some authors will refuse to admit that the small-flowered plants are *Lamarckiana*. They will insist that the true *Lamarckiana* is always large-flowered and that these variants are "mutants" or perhaps aberrant types. Yet the fact remains that the large- and small-flowered types are indistinguishable in taxonomic practise except for the bud and flower characters, and the writer can but believe that the large-flowered forms have been steadily selected by those who have for so many years carried *Lamarckiana* along to its present state.

Whether the small-flowered forms illustrate reversion towards a *biennis* type of flower is a matter worthy of critical attention. The behavior of my hybrids between

biennis and *grandiflora* so far tested in the F_2 generation (briefly described later in the paper) showed clearly that there is a segregation of flower size. Some types of flowers appeared in the F_2 that were even larger than the *grandiflora* parent species and as large as the largest *Lamarckiana;* others were smaller than the flowers of the F_1 hybrid parent, although none were so small as the *biennis* parent species. Between the large and the small-flowered F_2 hybrids was an apparently perfect range of intermediates.

Gates ('11*b*) has criticized the comparison of the flowers of my hybrid plants 10.30*La* and 10.30*Lb* to those of *Lamarckiana* (Davis, '11) on the ground that their measurements were too small (petals 2.2 cm. long), being unwilling to recognize the existence of small-flowered types of *Lamarckiana.* He apparently, however, fails to appreciate that the problem is only in part what may appear in the F_1 generation. It is the behavior of the hybrids in the second and later generations that will demonstrate the possibilities of the double organization of the F_1 hybrid, and the results of my cultures of the F_2 generation indicate that forms with flowers fully as large as those of the largest-flowered *Lamarckiana* may be readily obtained in abundance. It may prove more difficult to establish in the hybrids certain points of stem coloration and leaf form, but my later studies indicate that these results will depend chiefly upon proper discrimination in the choice of parents for the cross, especially among the large variety of biotypes included in *biennis.*

3. Further Races of *Œnothera biennis* L.

I have discarded for experimental purposes the races *biennis A* and *biennis B* which were used in the first crosses with *O. grandiflora* (Davis, '11). These have been supplanted by biotypes much more favorable for the purposes of the investigation. Among the American wild forms of *biennis* the best so far obtained is *biennis D*

of my cultures—a fairly large-flowered type (petals about 2 cm. long) with relatively broad leaves and a green stem the papillate glands[2] of which are colored red by anthocyan.

This form, *biennis D*, is widespread. It is common in the suburbs of Boston, and I have seen it at Woods Hole, Plymouth, and in the neighborhood of Philadelphia. There is considerable variation in the breadth of the leaves. Similar plants may also be found with clear green stems indicating that the red coloration of the glands may not always be a firmly established character in the strains that show it.

The plant which was the starting point of the strain *biennis D* grew wild in the grounds of the Bussey Institution in company with a number of similar types. From self-pollinated seed a culture of 51 plants was brought to maturity in the summer of 1911, all the plants being alike, even to the red coloration of the glands on the stems.

[2] Apparently there has been but little study of the surface tissues of *Œnothera* with reference to their secretory or glandular functions. However, former statements of the absence of external glands in the group *Onograriecæ* can not be accepted (see "Solereder's Systematic Anatomy of the Dicotyledons," Vol. II, p. 931, 1908). The forms of *Œnothera* with which I am working (*biennis, grandiflora, Lamarckiana*, etc.) have over the younger portions of the plants more or less of a somewhat sticky moisture, and the problem is from what cells do these secretions come. The hairs on these plants are of two types, both unicellular, (1) short hairs attached directly to the surface, (2) much longer and stouter hairs each arising from the top of a papilla. The papilla in section is seen to consist of a projection of the epidermis into which extends a number of hypodermal cells. These hypodermal cells in younger portions of the plant are filled with a dense viscous-like substance, as are also some of the epidermal cells. On old portions of the plants these cells, like those of the hypodermal tissue in general, are found to be quite empty. Thus the appearance of the contents of the cells composing the papilla indicates that it is secretory in function and I have consequently termed it a gland. The structure is important in experimental studies since its coloration in some forms follows that of the stem on which it lies (green or reddish) while in other forms the papilla may be colored red upon green stems and ovaries. We trust that the evidence presented above will justify the term gland which, if correct, is preferable to the designations papillæ, pustules, tubercles, red tubercle-like bases, red prickles, papillose based, red tuberculate, etc., that have been applied by my correspondents to this structure, or to the hair.

The original plant was crossed in 1910 with the strains *grandiflora B* and *D* (for descriptions see Davis, '11, pp. 205–207) and the interesting F_1 hybrids described in this paper were from this cross.

The chief characteristics of the strain *biennis D,* when under good cultivation, are as follows:

1. *Rosettes.*—Mature rosette (Fig. 1) about 4 dm.

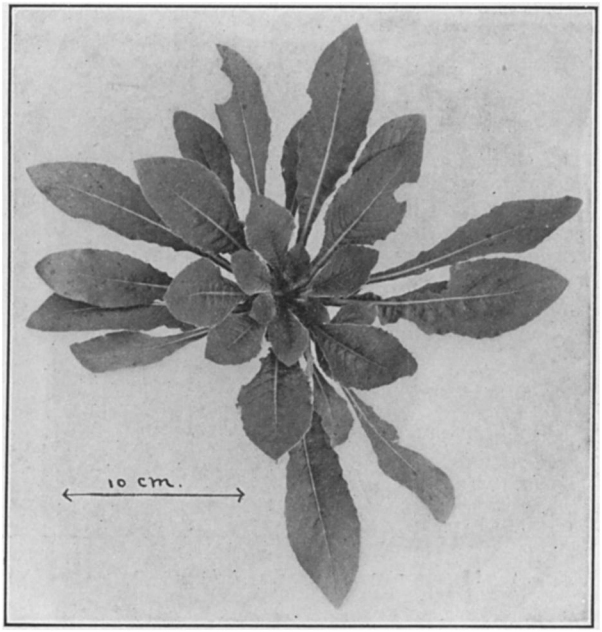

FIG. 1. Mature rosette of *Œnothera biennis,* D (11.13a).

broad. Leaves broadly elliptical, 2–2.5 dm. long, somewhat crinkled, margin sinuate, irregularly toothed and cut below, green with occasional red spots.

2. *Mature Plants.*—The mature plants (Fig. 2), 1–1.5 m. high, have long irregularly spreading side branches from the rosette and main stem; collar[3] at the base of the branches inconspicuous. Stems green above, punctate

[3] The term collar is suggested for the swollen ring at the base of the larger branches, which in some species of *Œnothera* (*e. g., grandiflora*) is very conspicuous.

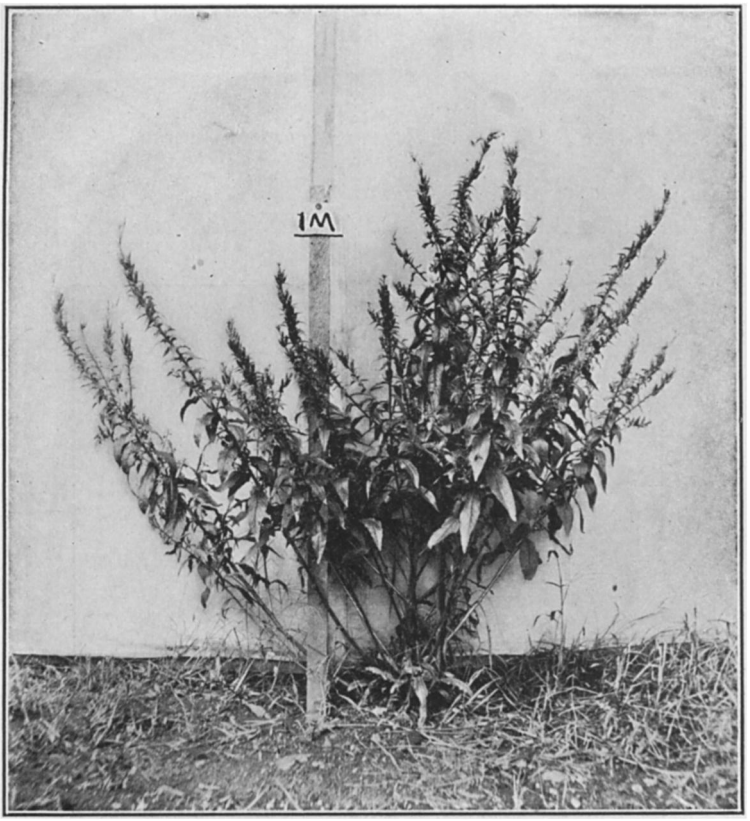

FIG. 2. Mature plant of *Œnothera biennis*, D (11.13a).

with red papillate glands at the base of long hairs. Basal leaves on the main stem elliptical (Fig. 3), about 17 cm. long, without marked crinkles, irregularly toothed; leaves above lanceolate.

3. *Inflorescence.*—Bracts lanceolate, $\frac{1}{3}$–$\frac{1}{2}$ length of buds (Fig. 3), frequently deciduous, leaving the fruiting branches destitute of leaves.

4. *Buds.*—About 5 cm. long, the cone 4-angled. Sepals green, pubescent with numerous long hairs arising from papillate glands among which are short hairs; sepal tips not markedly attenuate.

5. *Flowers.*—Fairly large (Fig. 3). Petals about 2

cm. long. Stigma lobes slightly below tips of anthers, 3 mm. long, pollinated in the bud. Papillate glands on ovaries red.

6. *Capsules.*—Gradually narrowing from the base, 2–2.5 cm. long.

7. *Seeds.*—Light brown.

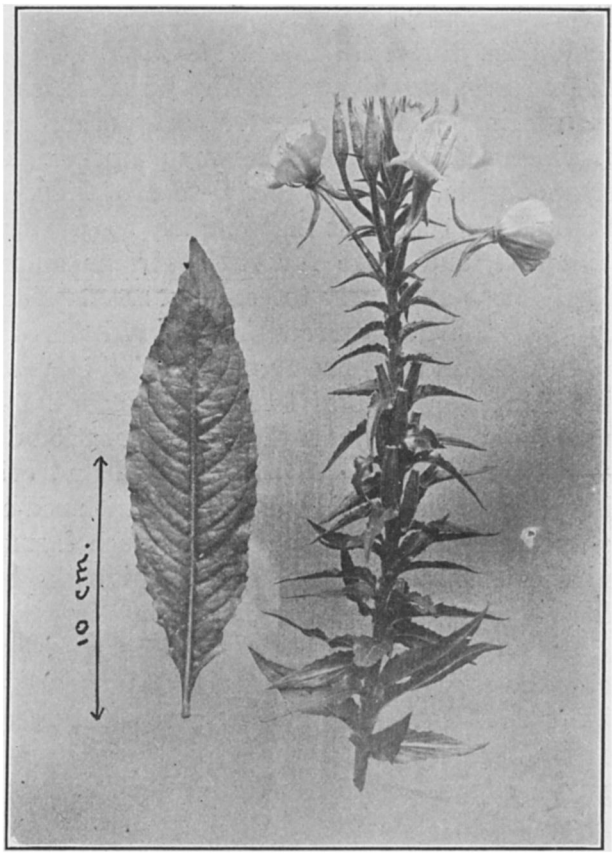

FIG. 3. Flowering side branch of *Œnothera biennis,* D (11.13a), with a leaf from the lower portion of the main stem.

A comparison of *biennis D* with the strains *biennis A* and *B* (Davis, '11, pp. 198–200) will show that it has certain important characters, present in *Lamarckiana,* which were not exhibited by those types of *biennis* first

employed in my crosses. The most important of these characters are (1) the stem coloration, green punctate with red papillate glands, (2) a broader rosette leaf somewhat crinkled, and (3) broader and larger foliage leaves. The flowers, half again larger than those of *biennis A* and *B,* are of a size more favorable to give large-flowered hybrids approaching *Lamarckiana* when *grandiflora* is employed as the other parent of the cross.

Although the differentiation of *biennis D* as a biotype has marked a great advance in the possibilities of my experimentation, there are undoubtedly other races of *biennis* which will prove better for my purposes. Thanks to correspondents, I have received seed of *biennis* from England and the continent that is likely to give types of great interest, and more favorable strains among the American forms are likely to come to hand. There are some beautiful large-flowered English and Dutch forms of *biennis* which in their broad and crinkled leaves and in their habit are very similar to *Lamarckiana,* but I have not as yet found among them the stem coloration desired. However, it is to be expected that broad and crinkled-leaved types of *biennis* will be discovered with red-colored glands upon the stems and ovaries and such forms when crossed with *grandiflora* are likely to give the hybrids most like *Lamarckiana.* It will take some time to differentiate such strains, but they are certain to exist since these characters are presented in part by various types of *biennis.* Indeed they seem likely to prove not uncommon judging from the collections that have come to me during the past year from botanists who have kindly interested themselves in the problem.

In my previous paper (Davis, '11, p. 201) mention was made of a southern *Œnothera* (strain *S*) which appeared in cultures from the wild seed collected by Tracy as *Œnothera grandiflora.* Further studies have shown that the plant is annual, its rosette being small and transitory as in *grandiflora.* The form has been described and named *Œnothera Tracyi* by H. H. Bartlett ('11). I have

crossed this species with *grandiflora,* but the hybrids were very far from *Lamarckiana* chiefly since the hybrids had narrow leaves and lacked the persistent rosettes associated with the usual biennial habit of the latter plant. The results show clearly that any form crossed with *grandiflora* to produce *Lamarckiana*-like hybrids must be one with large persistent rosettes such as are presented by the northern types of *biennis.*

4. Further Races of *Œnothera grandiflora* Ait.

A type of *Œnothera grandiflora* appeared in my cultures of 1910 (Davis, '11, p. 204), first noted because of its rosette of green, much crinkled leaves. This strain, *grandiflora I,* was further cultivated in 1911 but has proved less favorable for my purposes than the strains *A, B,* and *D,* chiefly for the reason that its leaves have a pronounced petiole and the stems bear towards their tips dense clusters of flowering side shoots. The strain *grandiflora I* is a well-defined type very different from the much more common forms of *grandiflora* and as such is of interest.

To add to the data on the composition of *Œnothera grandiflora* as it grows wild (Davis, '11, pp. 202–205) a culture of 169 plants from wild seed, collected by Tracy at Dixie Landing, Alabama, in 1907, was brought to maturity during the season of 1911. In this culture 42 plants proved to be *Œnothera Tracyi* referred to above, 9 plants were unmistakably of the strain *grandiflora I,* and the remaining 118 plants were close to the strains *grandiflora A, B,* and *D,* which it will be remembered are so similar as to be essentially of one type.

It seems clear from my cultures of wild seeds, a total during the past four years of about 300 plants, that the prevailing form of *grandiflora* is that represented by the strains *A, B,* and *D,* previously described (Davis, '11, pp. 205–207). There is a range of variation, chiefly in the breadth of the leaves, and these strains (*A, B,* and *D*) are

the broader-leaved, more luxuriant forms such as the gardener would be likely to select for cultivation.

5. HYBRIDS IN THE F_1 GENERATION FROM THE CULTURES OF 1911

The most important cultures of 1911 in the F_1 generation were those of the following combinations of parent species.

1. *grandiflora B* × *biennis D* (11.35).
2. *grandiflora D* × *biennis D* (11.32).
3. *grandiflora I* × *biennis D* (11.37).
4. *grandiflora D* × *Tracyi* (11.33).

Of these the most interesting, with respect to the resemblance of some of its plants to *Lamarckiana,* was the first culture in the list—*grandiflora B*×*biennis D* (11.35). The greater part of this account will consequently be devoted to this culture, but the others will be

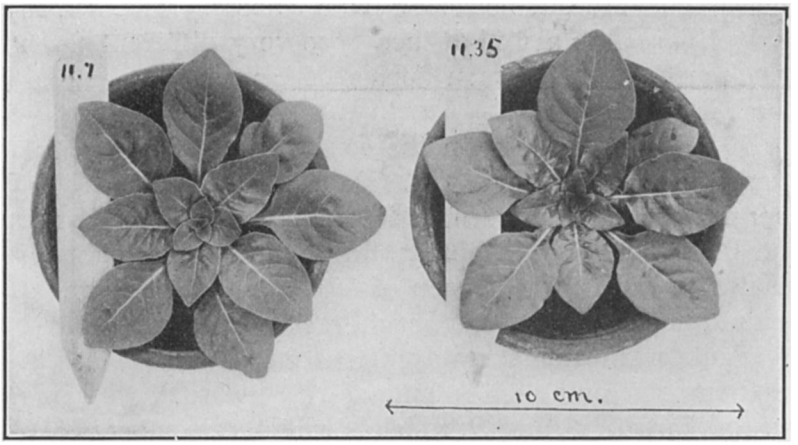

FIG. 4. Young rosette of an F_1 hybrid *grandiflora* B × *biennis* D (11.35), compared with that of *Lamarckiana* Z (11.7).

briefly described, chiefly with reference to the coloration of the papillate glands in which all four cultures agree in exhibiting a behavior that was not to be expected.

1. *grandiflora B* × *biennis D* (11.35).—This culture

was derived from the contents of two capsules containing about 300 seeds. From these 247 seedlings appeared in the pans within six weeks; 198 young rosettes were potted and finally 180 large rosettes were set out. Thus, in reducing the culture, 67 smaller rosettes which gave less promise of developing into vigorous plants were discarded.

The similarity of the young rosettes of the hybrid to those of *Lamarckiana* is shown in Fig. 4. The mature

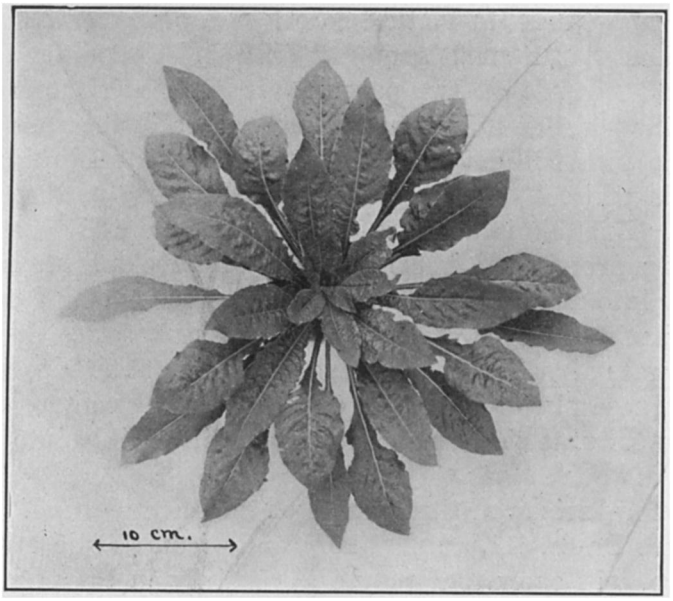

FIG. 5. Mature rosette of the F_1 hybrid 11.35a, *grandiflora* B × *biennis* D; representative of the mass of the culture.

rosettes (Fig. 5) presented characters that were clearly blends in various degrees between the parent types, blends very hard to define because of a range of variation in the leaves. The leaves were conspicuously crinkled as in *Lamarckiana,* differing from the latter types of my cultures chiefly in being more deeply toothed and cut at their bases and in being colored a slightly darker shade of green with more numerous reddish spots

of anthocyan. The rosettes resembled those of *Lamarckiana* very closely in their morphology, much more closely than those of my earlier hybrids (see Davis, '11, Figs. 9 and 12), as would be expected from a cross involving the broader-leaved biotype *biennis D*.

Continued experience with the rosettes of *Œnothera* has shown that there is a greater range of variation among the F_1 hybrids of the species crosses than might be expected by those whose studies have been upon hybrids of closely related races. It has always been possible to arrange the mature rosettes of a large culture in a series with a small group at each end that differ and incline towards the two parents. It is my method to set out the rosettes in this order and generally the rosettes at the ends will develop into mature plants exhibiting a similar degree of divergence from the mass of the culture. But this is by no means a fixed rule and rosettes giving promise of a certain line of development may grow into mature plants of an unexpected type. I have emphasized the divergence between the rosettes at the extremes of an F_1 generation, but it must not be supposed that these plants constitute classes. On the contrary, in large cultures they apparently range insensibly into the mass.

A comparison of the parents of this cross, *grandiflora B* (see Davis, '11, pp. 205–207) and *biennis D* described above, will show that they differ chiefly in the relative form, size and proportions of their organs, and their characteristics would be expected to be present in the F_1 hybrids in blended relations, as is the fact. The only character observed that might be sharply contrasted as present or absent in these parents is the coloration of the papillate glands which in *biennis D* are red on green stems and ovaries, and in *grandiflora D* are uncolored, *i. e.*, lack the red. The hybrids of the F_1 generation naturally might be expected to exhibit either the presence or absence of the red coloration in these glands as one or the other condition might be dominant.

To my surprise, with the appearance of the main stem from the rosettes two classes of plants became at once defined in the culture of these F_1 hybrids: Class I, represented by 12 plants with red glands on the ovaries and the green portions of the stems, and Class II, represented by 168 plants in which the papillate glands similarly situated lacked the red coloration. There were apparently no intermediates, with respect to this character of gland coloration, between these two well-defined types which usually differed in other respects, as noted below.

The mature plants of the two classes could generally be sharply contrasted with one another in the following respects.

Class I (12 Plants)	Class II (168 Plants)
Papillate glands colored red on the ovaries and green portions of the stems.	Red coloration absent from the papillate glands on the ovaries and green portions of the stems.
Mature plants (Fig. 6) symmetrical in habit, collar at base of the long branches inconspicuous.	Mature plants (Fig. 8) somewhat straggling in habit, collar at base of the long branches more conspicuous.
Lower leaves (Fig. 7) narrowly elliptical, lanceolate on upper portions of the plant.	Lower leaves (Fig. 9) broadly elliptical, ovate on upper portion of the plant.
Bud cones 4-angled.	Bud cones round in section.
Stigma lobes 6–7 mm. long.	Stigma lobes 3–4 mm. long.
Capsules about 3.3 cm. long.	Capsules about 2.3 cm. long.
Bracts persistent, developing on fruiting branches into lanceolate leaves.	Bracts deciduous, the fruiting branches becoming nearly or wholly destitute of leaves.

We have said above that the two classes of plants could usually be sharply contrasted with respect to the characters listed. There were found no exceptions as regards the coloration of the glands, but with respect to the other characters some variation was exhibited, especially among the large number of plants in Class II. For example, the plant of the culture most resembling *Œnothera Lamarckiana* (11.35*La*, to be described later) was representative of Class II in all respects except that

it had 4-angled buds. Class II was more diversified than Class I, but this was probably because of its being represented in the culture by fourteen times as many plants.

It is of interest to compare the hybrids of these two classes with their parents. The plants of Class I resemble the *biennis* parent in the red glands on ovaries and green stems, inconspicuous collars, narrower leaves, and 4-angled buds; they resemble the *grandiflora* parent in having longer stigma lobes, longer capsules and persistent bracts. The plants of Class II resemble the *grandiflora* parent in the absence of the red coloration in the glands on the ovaries and green portions of the stems, and in having more conspicuous collars, broader leaves, and round buds; they resemble the *biennis* parent in having shorter stigma lobes, shorter capsules, and deciduous bracts. It will be noted that the contrasted characters are mixed for both classes of plants, some of them being *biennis*-like and some of them *grandiflora*-like. Thus neither class could be claimed as patroclinous or matroclinous except it were established that gland coloration, size of collar, form of leaves and form of buds are more important specific characters than length of stigma lobes, length of capsule and the persistent or deciduous nature of the bracts, or vice versa. Of these diverse characters who would be willing to express the opinion that one set or the other is not of consequence in a description of the forms? Yet it would be necessary to disregard one or the other set of characters if either class of hybrids were defined as patroclinous or matroclinous.

We have stated before that the plants of the culture were set in the ground with the few more *biennis*-like rosettes at one end of the bed, and the few more *grandiflora*-like at the other end, and that these extreme forms graded insensibly into the mass of the culture. It is important to note that not one of the 12 plants of Class I was at either end of the series, but they were scattered irregularly through the culture, in some cases 2 or 3 close together, but usually wide apart. I know as yet no way in

which the representatives of Class I can be distinguished in the rosette condition; they certainly do not constitute either of the small groups of rosettes that are more like one or the other of the two parents.

The differentiation of these two classes of hybrids in an F_1 generation appears to be similar to the phenomenon of "twin hybrids" reported by De Vries ('07) although the contrasted characters are somewhat different. The behavior is most interesting, especially in its relation to the results expected in F_1 generations according to gen-

FIG. 6. Mature plant of the F_1 hybrid 11.35m, *grandiflora* B × *biennis* D; representative of Class I.

eral experience and Mendelian laws. We should have expected the red coloration of the papillate glands of the *biennis* parent to be either dominant or recessive to the uncolored glands of the *grandiflora* parent; as a matter

of fact, both conditions appeared with apparently no intermediates. It would, however, be unsafe to regard this behavior as an exception to Mendelian laws since there is the possibility that the strain *biennis D* is heterozygous with respect to the red coloration of its papillate glands. To be sure, a culture of 51 plants from self-pollinated seed of the wild plant (*biennis D*) were uniform even to the red coloration of the glands on the green stems and ovaries, but, nevertheless, there are similar wild *biennis* types that lack this coloration, and there has

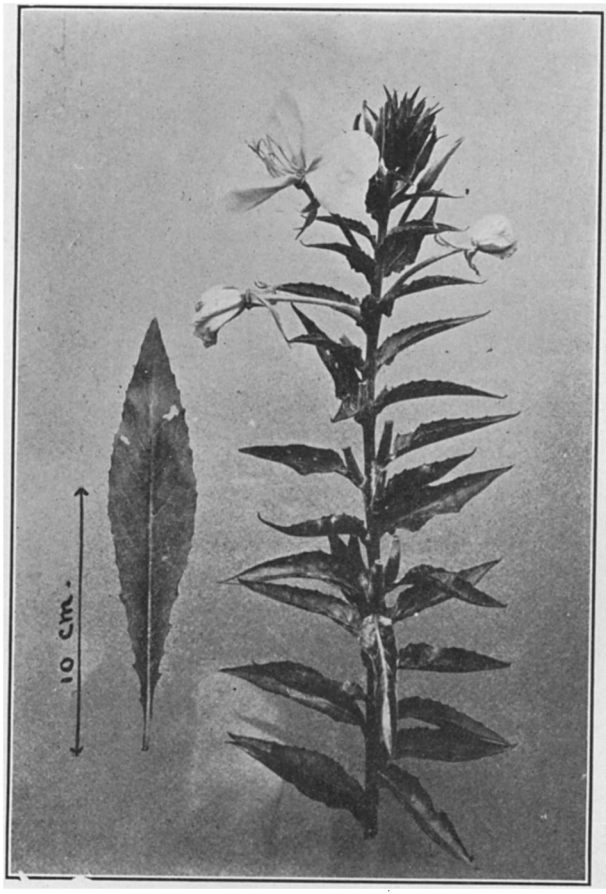

FIG. 7. Flowering side branch of the F_1 hybrid 11.35m, *grandiflora* B × *biennis* D; representative of Class I. At the left is a leaf from the lower portion of the main stem.

not yet been time to determine whether or not the strain *biennis D* is homozygous in all of its characters. The matter has no especial bearing on the immediate purposes of my studies, but will become vital to further investiga-

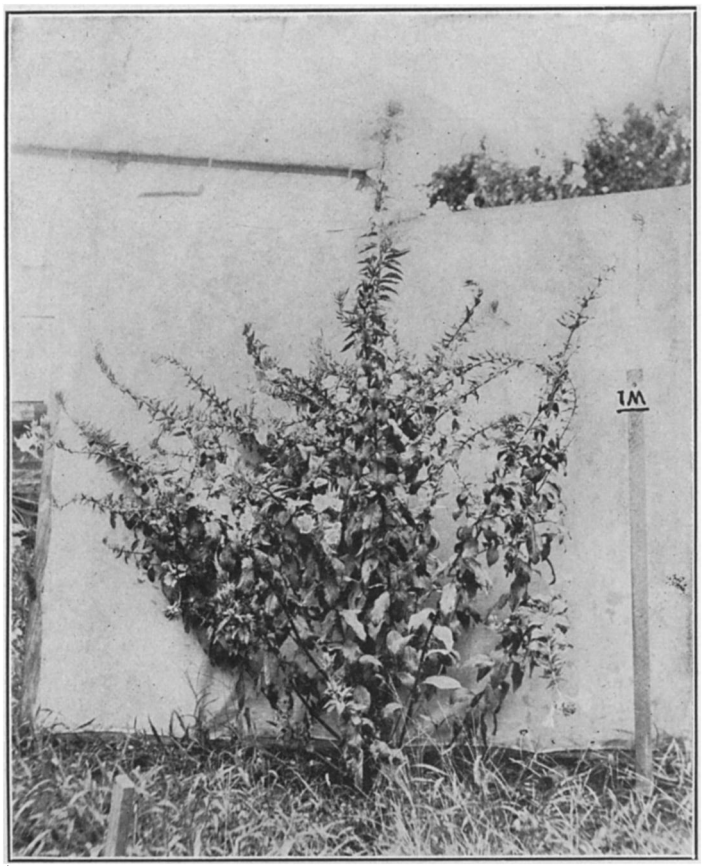

Fig 8. Mature plant of the F_1 hybrid 11.35a, *grandiflora* B × *biennis* D; representative of Class II.

tions on the behavior of the characters of gland coloration in the Œnotheras.

My plans for the further study of these hybrids in second generations involve cultures in pure lines from representative plants of both Class I (11.35*m*, Figs. 6 and 7) and Class II (11.35*a*, Figs. 8 and 9), and cultures

of reciprocal crosses between these same representatives (11.35$m \times a$ and 11.35$a \times m$). The results of the reciprocal crosses will be awaited with especial interest in view of De Vries's ('11) recent paper on double reciprocal crosses. Furthermore, a large second generation will be grown from self-pollinated seeds of a plant (11.35La) in Class II selected as being in more respects similar to

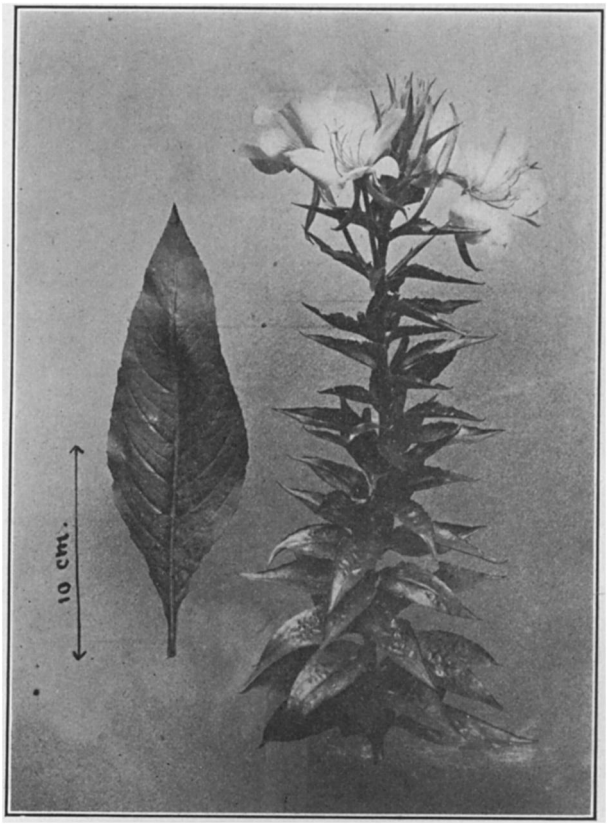

FIG. 9. Flowering side branch of the F_1 hybrid 11.35a, *grandiflora* B × *biennis* D; representative of Class II. At the left is a leaf from the lower portion of the main stem.

Lamarckiana than any hybrid of my crosses so far grown.

Let us consider the hybrids just described with respect to their resemblance to *Lamarckiana*. As regards the

size of the flowers both classes of hybrids presented essentially the same types (petals 2.5–2.7 cm. long), closely intermediate between those of the parents, but the flowers of Class I were similar to those of *Lamarckiana* in the length of the stigma lobes, in their 4-angled buds and in having red glands on their ovaries. In the form and size of the capsules and in the deciduous habit of the bracts Class II had the advantage of a closer resemblance to *Lamarckiana*. In the broader and somewhat crinkled leaves the advantage was very much in favor of the plants in Class II, but in the coloration of the glands on the stem the plants of Class I were *Lamarckiana*-like. The situation seems mixed and we must await the outcome of the cultures as planned above in the hope of some interesting conclusions. The writer in selecting a representative of Class II (11.35*La*) as more like *Lamarckiana* than any other plant has laid the greater emphasis upon the characters of foliage and buds (in this plant stout and 4-angled), believing that these characters of *Lamarckiana* are more important and more difficult to obtain in hybrids than many of the others.

A description of this plant (11.35*La*), the F_1 hybrid, so far obtained, most closely resembling *O. Lamarckiana*, will now follow, arranged to bring out its important characteristics in comparison with those of the parent species and with *Lamarckiana*.

Hybrid 11.35*La*

1. *Rosette.*—The rosette of this plant was unfortunately mutilated by cut worms which destroyed the central bud so that the shoots that grew to bear flowers were all from side buds. The rosette, however, was similar to that shown in Fig. 5 (11.35*a*), that is to say, it was representative of the mass of the culture. My attention was first attracted to this plant by the greater length and breadth of the leaves upon the young side shoots and their conspicuous crinkling, well illustrated in Fig. 10. As the

side shoots elongated (Fig. 11) the foliage retained the promise of the earlier condition (Fig. 10), the shoots bearing leaves distinctly larger and more crinkled than the other plants of Class II, but of the same form.

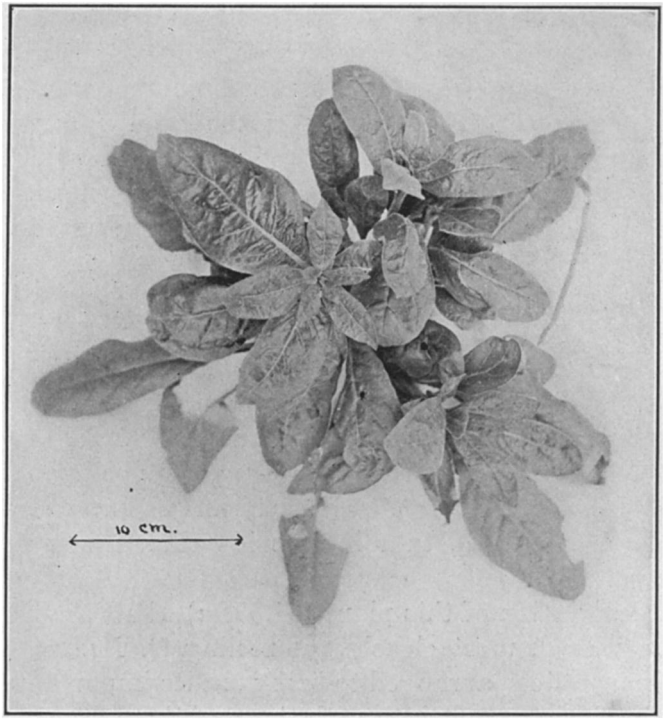

FIG. 10. Rosette of the F_1 hybrid 11.35La, *grandiflora* B × *biennis* D. Central bud destroyed by cut worms; side shoots developing with large, crinkled, *Lamarckiana*-like leaves.

2. *Mature Plant.*—Five strong side shoots, 1.1–1.3 m. high, (Fig. 12) developed from the mutilated rosette. Their stems were green above, reddish below, the papillate glands following the coloration of the stem, *i. e.*, they were green (uncolored) on the ovaries and younger portions of the stem as in Class II, and not red as in my cultures of *Lamarckiana*. The basal leaves, about 23 cm. long, were broadly elliptical and strongly crinkled, the margins scarcely toothed except at the base;

the leaves above were ovate or broadly elliptical; all leaves had short petioles. The leaves on the plant throughout the history recorded by Figs. 10, 11 and 12 were so similar in form, size, and texture to those of my cultures of *Lamarckiana* that I do not believe the plant could have been easily separated by its foliage if grown among them. The absence of red colored glands on the green stem and ovaries appeared to the writer to be the only character of importance distinguishing it from

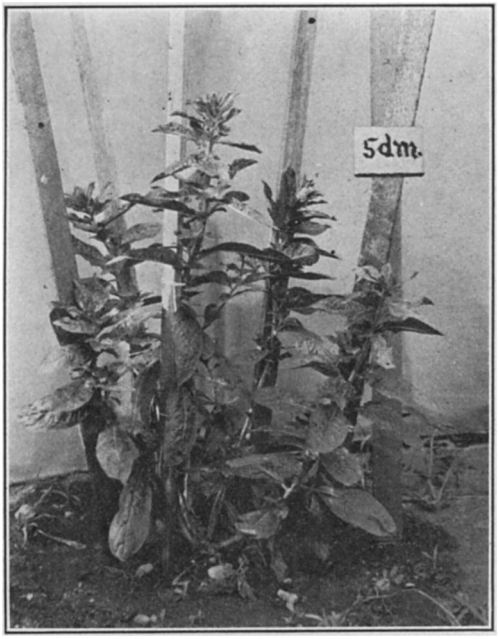

FIG. 11. Young plant of the F_1 hybrid 11.35La, *grandiflora* B × *biennis* D, showing *Lamarckiana*-like foliage of large, crinkled leaves.

Lamarckiana during its development up to the time of flowering.

3. *Inflorescence.*—The inflorescence (Fig. 13) presented shorter internodes than is characteristic of *Lamarckiana* as I have observed it, and was in consequence more flattened at the top, in this respect resembling the inflorescence of *gigas*. The bracts were similar

in form to those of *Lamarckiana,* but were not quite so closely sessile.

4. *Buds.*—The buds, about 7 cm. long, were strongly 4-angled (in this respect departing from the rule in Class

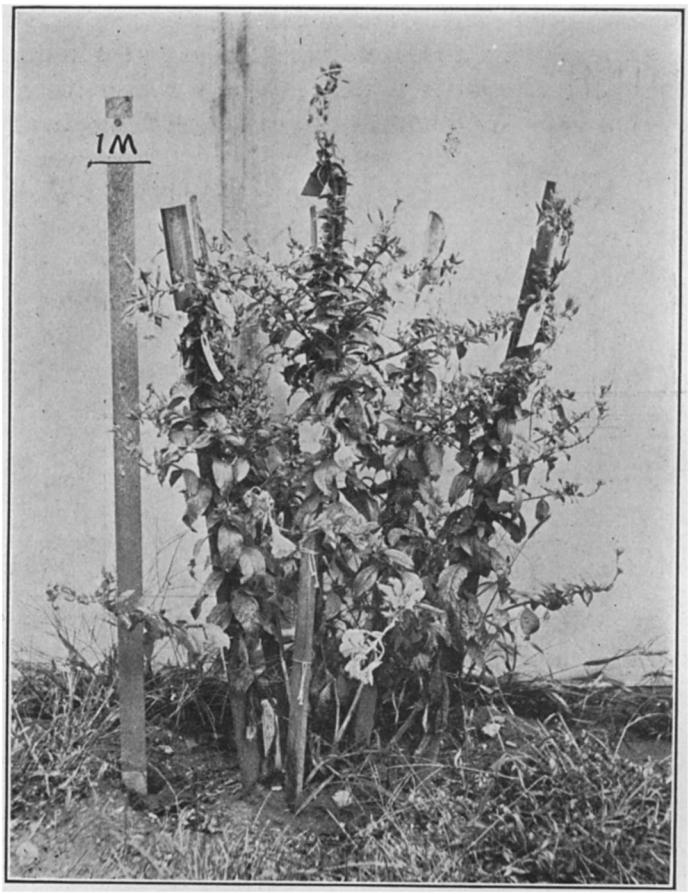

FIG. 12. Mature plant of the F_1 hybrid 11.35La, *grandiflora* B × *biennis* D, with *Lamarckiana*-like foliage.

II). Their pubescence was like that of *Lamarckiana,* long hairs arising from papillate glands among much shorter hairs which were, however, less numerous than in *Lamarckiana.* The sepals were green, their tips of medium length, not markedly attenuate as in *grandiflora;*

the cone of the bud was stout. The strong resemblance of the buds in form to those of *Lamarckiana* was an important point in the choice of this plant as favorable for further cultivation. They were, however, from 2–3 cm. shorter than those of the large-flowered *Lamarckiana,*

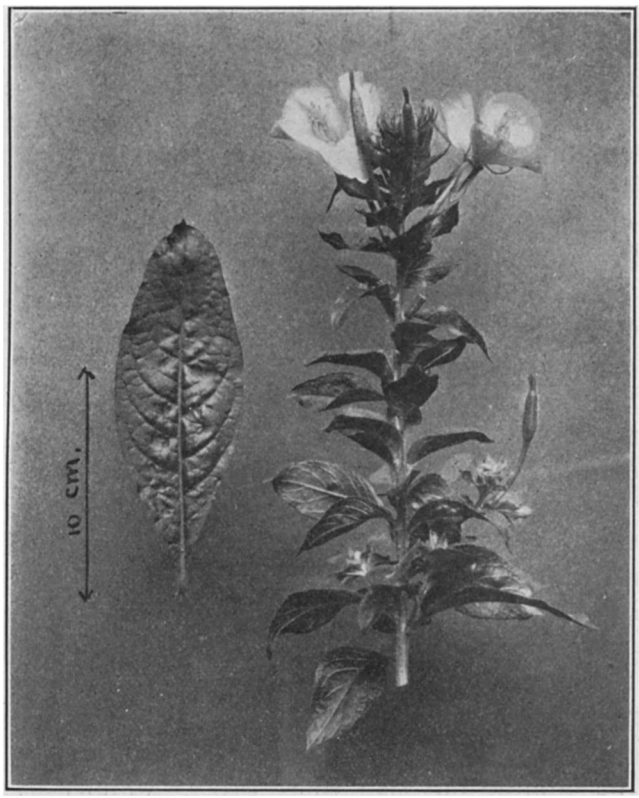

FIG. 13. Flowering side branch of the F_1 hybrid 11.35La, *grandiflora* B × *biennis* D. At the left is a leaf from the lower third of a side stem.

but as large as the small-flowered type. There should be little difficulty in obtaining larger sizes in the F_2 generation.

5. *Flowers.*—The flowers were medium-sized, petals about 2.5 cm. long. The stigma lobes, 3–4 mm. long, were slightly below the tips of the anthers. Ovaries were

green (without red-colored glands). The flowers were indistinguishable from those of the small-flowered types of *Lamarckiana* except for the absence on the ovaries of red-colored glands. They differed from the large-flowered types (petals 4–4.5 cm. long) not only in size, but also in the shorter stigma lobes which were not above the tips of the anthers. My experience has shown that the flowers in a second generation of these *Œnothera* hybrids will present a wide range in their measurements, many flowers surpassing in size those of the *grandiflora* parent and equalling the largest forms of *Lamarckiana*. Consequently there is good reason to be hopeful of obtaining large-flowered types in the F_2 generation.

6. *Capsules.*—The capsules, about 2 cm. long, were stout and similar to those of *Lamarckiana*. The bracts exhibited the deciduous tendencies of Class II, a *Lamarckiana*-like habit.

7. *Seeds.*—The seeds were of a shade of color intermediate between the light and dark brown of the parents.

Speculation as to the probabilities of the behavior of these F_1 hybrids (11.35*La*, 11.35*a*, and 11.35*m*), and the reciprocal crosses (11.35 $a \times m$, and 11.35 $m \times a$) in the second generation, is not called for, since we hope in a few months to have data on the questions involved. Some of these problems concern the general behavior of species crosses and are to the writer as interesting and important as the attempt to synthesize a *Lamarckiana*-like hybrid between *biennis* and *grandiflora*. It may, however, be pointed out that there are no important characters of *Lamarckiana* which are not represented in some one of these hybrids or in their parents, except those of the somewhat larger size of certain organs. Since my cultures have shown that hybrids of *Œnothera* in the F_2 generation frequently present marked advances over their parents in the measurements of petals, leaves, etc., these differences of size in an F_1 hybrid are likely to prove more apparent than real.

2. *grandiflora* $D \times$ *biennis* D (11.32).—As would be

expected, this cross was very similar to that just described (11.35, *grandiflora B* × *biennis D*). The 195 plants brought to maturity fell into two classes distinguished by precisely the same groups of characters as in the previous cross. Class I with red papillate glands, etc., was represented by 11 plants; Class II by 184 plants. Certain small differences of foliage, apparent only on close inspection, together with the fact that no plant seemed as favorable for my purposes as 11.35*La,* determined the selection of the other culture (11.35) as the one upon which to base further studies.

3. *grandiflora I* × *biennis D* (11.37). The rosettes of this cross (Fig. 14) were more like those of *Lamarckiana*

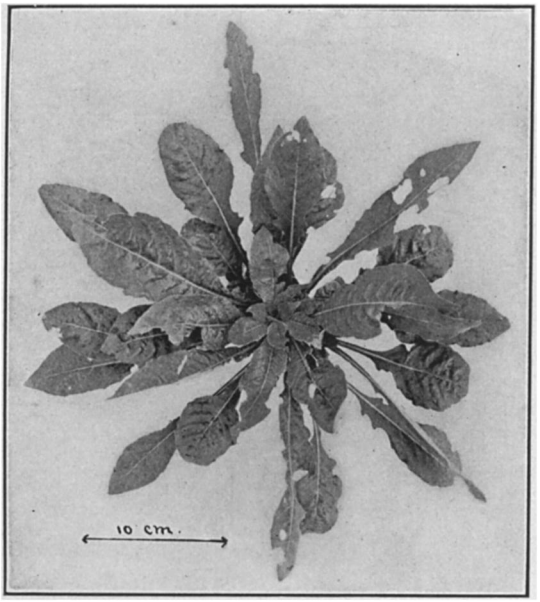

FIG. 14. Mature rosette of an F_1 hybrid, *grandiflora* I × *biennis* D (11.37). A type of rosette very similar to that of *Lamarckiana*.

than in any of my hybrids so far obtained, but the mature plants were disappointing. The strain *grandiflora I* has a strongly petioled leaf, and the stems bear dense clusters of flowering side shoots. These characters appeared in

pronounced form in the F_1 hybrids rendering them less favorable for my studies than the crosses with the strains *grandiflora B* or *D*. The culture was interesting since it presented two classes of F_1 hybrids as in cultures 11.35 and 11.32, separated by identical groups of characters. Out of 144 plants brought to maturity 4 plants were of Class I and 140 plants were of Class II.

A culture of the reciprocal cross of these types, *biennis D* × *grandiflora I* (11.39), was started late in the season after the appearance of the paper of De Vries ('11) on double reciprocal crosses, but the culture was unable to reach maturity. By September 15, out of a total of 171 rosettes, 31 had sent up side shoots of sufficient length (2–4 dm.) to determine the coloration of the papillate glands; 21 of these plants had red glands on a green stem, and in 10 plants the glands were uncolored.

4. *grandiflora D* × *Tracyi* (11.33).—The F_1 hybrids of this cross were remarkable plants 2.5–3 m. high, developing from transitory rosettes and with a luxuriant foliage of narrow leaves. As noted before, the plants were very far from *Lamarckiana*. It is a cross that is likely to occur in nature where the two species grow together as at Dixie Landing, Alabama, and this possibility must be reckoned with in analyses of the two species. *Œnothera Tracyi* has the red coloration of the papillate glands characteristic of *biennis D*. The 40 plants of the culture fell sharply into two classes distinguished by characters similar to those of the crosses between *biennis D* and *grandiflora*. There were 3 plants of Class I, with red glands, etc., and 37 of Class II.

The cultures of the F_1 hybrids as recorded above have given data that can not be satisfactorily discussed until similar crosses have been made with parents that are beyond question homozygous. My strains *grandiflora B* and *D* have now been carried in pure line for three generations and have proved uniform, but the parent plants *biennis D* and *Tracyi* were from wild seed. It does not seem probable that the latter types are heterozygous, for

the reason that both have the habit of close pollination, but, nevertheless, the forms are not above suspicion. As the data stand the most interesting features shown by the F_1 hybrids are: (1) The absence of dominance on the part of any character of the parents, including the coloration of the papillate glands, (2) the sharp differentiation of two classes of hybrids (twin hybrids?) clearly distinguished by groups of characters, and (3) the absence of marked patroclinous or matroclinous conditions in either of these two classes. The differentiation of two classes of hybrids has been a new phenomenon in my experience with F_1 hybrids of *Œnothera* which have previously proved remarkably uniform except for the extremes represented by a few plants somewhat approaching the respective parents.

6. Hybrids in the F_2 Generation from the F_1 Hybrid Plants 10.30*La* and 10.30*Lb*.

In my paper of a year ago (Davis, '11, pp. 211–217) two plants were described and figured which among the hybrids up to that time most closely resembled *Œnothera Lamarckiana*. They were the F_1 hybrid plants designated 10.30*La* and 10.30*Lb*, the result of the cross *grandiflora B* × *biennis A*. Cultures from self-pollinated seed of these two plants were grown in the season of 1911, giving considerable data on the behavior of such a cross in the second generation.

These F_2 hybrids were not grown with the expectation that any of the plants would be taxonomically the same as *Lamarckiana*, since it had been apparent for some months that the strain *biennis A* is not so favorable a biotype as many others to cross with *grandiflora* in the hope of obtaining *Lamarckiana*-like hybrids. The hybrids were grown to satisfy my keen desire to observe the behavior of such a cross and in the hope that its study would prove helpful in the planning of future work.

The mass of the F_2 hybrids held in their characters

within a certain wide range of variation, but a large number of very striking types appeared, which, if they come reasonably true in a third generation, must be regarded as new forms of specific rank. These variants constituted in some cases classes entirely distinct from all of the other plants and in other cases were extreme types of segregates connected by intermediate forms more or less closely with the mass of the hybrids. Although the two F_1 parent plants of these cultures, (10.30*La* and 10.30*Lb*) were sisters, their progeny was far from similar. Each F_1 hybrid plant gave rise to its own peculiar set of variants and the character of the plants constituting the mass of the cultures likewise differed greatly. The present description of the cultures will be very brief, but a more full and illustrated account may follow if studies of the more interesting of the variants in a third generation make the publication of such an account seem desirable.

1. THE F_2 GENERATION (11.41) FROM THE HYBRID PLANT 10.30*La*

This culture was grown from the contents of 26 capsules containing in all about 3,300 seeds. From these 1,505 seedlings appeared in the pans within 5–7 weeks, and 1,451 grew into well-developed rosettes. It was possible to select from the rosettes a group of 141 which were smaller than the average and had strongly etiolated leaves; these rosettes developed a peculiar group of dwarfed plants apparently constituting a clearly defined class in the culture. The mass of the rosettes presented a remarkable range, from many that resembled very closely the rosettes of *grandiflora,* distinguished by having broader leaves cut at the base (see Davis, '11, Fig. 6), to much smaller rosettes with narrow leaves somewhat *biennis*-like in form. Between these extremes was an assemblage of intermediates so various in character that I was unable to make any satisfactory classification further than the selection of the 141 etiolated

types. It may be said, however, that the green rosettes inclined more towards the female parent of the cross, *grandiflora B,* than towards the male, *biennis A.* The plants set in the ground numbered 1,310 green rosettes and 141 etiolated.

The green rosettes were set out with the more *grandiflora*-like at one end and the more *biennis*-like at the other. In general the plants at the more *grandiflora*-like end were at maturity considerably larger 1.5–2 m. high, than those towards the more *biennis*-like end where a small group of plants (about 20) remained small, 6 dm.–1.2 m. high. Among the larger plants were many (about 50) with flowers as large as or larger than those of the *grandiflora* parent, but these could not be separated as a class since the variation on individual plants was considerable and they intergraded with great perfection into the mass of the culture. The smaller plants at the more *biennis*-like end were variable, but all had flowers 2–4 times larger than those of the *biennis* parent. The larger flowers had petals measuring 4–4.5 cm. long (those of *grandiflora B* are about 3.3 cm. long); these flowers were therefore as large as the largest-flowered types of *Lamarckiana.* The large flowers also, as a rule, presented the *grandiflora* relation of the stigma to the anthers, *i. e.,* the stigma lobes were well above the tips of the anthers. Thus in flower size the culture showed a clear segregation, but inclined markedly towards the larger size of the *grandiflora* parent, in many cases surpassing this flower in its measurements.

The foliage of the culture presented a range of variation that defied my attempts at classification. That of some of the larger-flowered plants closely resembled *grandiflora,* and a few of the smaller plants had a foliage approaching that of *biennis,* but the leaves throughout the culture as a whole were larger than those of the parents of the cross and generally distinctly crinkled.

The mass of the culture was fairly close to the F_1 hybrid plant, 10.30*La,* from which it came, but there was

much variation from this plant in the measurements and proportions of the organs with the greater tendency towards the *grandiflora* parent type. There was, however, no constancy apparent and plants with flowers similar to *grandiflora* might present a foliage of a different type, most frequently in the form of larger and strongly crinkled leaves. A few plants were so close to *grandiflora* that taxonomically they would probably be included in the range of this species, although they could not be considered identical with the strain *grandiflora B;* there were no segregates so close to *biennis A*.

A count was made with the assistance of Mr. H. H. Bartlett of the plants with green stems (*biennis*-like) and of those with red stems (*grandiflora*-like). This was extremely difficult for the reason that the mass of the culture presented intermediate or mottled conditions similar to the F_1 hybrid plant 10.30*La*. However, of the 1,310 plants, 195 were classified as red-stemmed and 192 as green-stemmed; the red were markedly nearer the more *grandiflora*-like end of the culture and the green nearer the *biennis* end. The expected number according to simplest Mendelian ratio should have been about 327 plants of each color. Considering that anthocyan is so variable in its appearance, and consequently most unsatisfactory for a study of color inheritance, these results are by no means against Mendelian expectations. No two persons independently would make the same count in such a culture, for the results all depend upon what conditions of the plant are defined as intermediate or mottled.

The culture presented a number of remarkable types which no taxonomist would think of identifying with either *biennis, grandiflora,* or the F_1 hybrid plant 10.30*La*. These will not at present be described in their many characteristics, but will be briefly listed.

11.41*c*, a type with conspicuously crinkled leaves, represented by about 170 plants, apparently intergrading with other forms of the culture.

11.41*bk*, represented by 13 plants, narrow leaves, flowers very light yellow, anthers sterile.

11.41*s*, a single plant similar to 11.41*bk* except for broader leaves.

11.41*r*, representing the class separated as 141 etiolated rosettes. The plants of this class as they developed gradually outgrew the etiolated conditions of the younger stages, but remained dwarf. They varied greatly among themselves in flower size and foliage, and a dozen different types could have been selected.

The above list does not include the numerous types of segregates which clearly differed specifically from the F_1 hybrid plant 10.30*La*. These forms, being of hybrid origin, are of course marked segregates presenting in varying combinations and degrees characteristics of the parents of the cross, *biennis* and *grandiflora*.

2. The F_2 Generation (11.42) from the Hybrid Plant 10.30*Lb*

From the contents of 5 capsules, containing about 1,200 seeds, a culture of 992 seedlings appeared in the pans within 6–8 weeks and grew into well-developed rosettes. From these a group of 147 rosettes were readily selected for their uniformly small size and narrow leaves. They gave rise to a definite class of dwarfs which remained absolutely distinct from the rest of the culture; the plants were not etiolated or in other respects similar to the class of dwarfs separated in the culture (11.41) from the sister hybrid, 10.30*La*. The rosettes constituting the mass of the culture presented a wide range of form similar to that shown in 11.41, with the extremes approaching the rosettes of the *biennis* and *grandiflora* parents. Between the extremes was the similar large assemblage of intermediates of varying degrees which made a classification of the rosettes impossible. There was apparently no clearly marked tendency of the rosettes in the culture as a whole to resemble either of the parents.

The plants set in the ground numbered 833 large rosettes and 90 of the dwarf. This culture at maturity proved much more varied than the former (11.41). The plants at the more *grandiflora*-like end of the culture were in general considerably larger (1.7–2 m. high) than those at the *biennis* end (1–1.5 m. high), but there were many exceptions to the rule. The foliage proved exceedingly diverse. Occasional plants (about 20) presented a foliage similar to *grandiflora* although never matching the parent strain in all respects; a smaller number of plants (about 5) presented a foliage resembling that of *biennis*. The foliage, excluding the exceptional types, ranged from lanceolate leaves to broadly elliptical or ovate leaves with well defined crinkles.

Many plants bore flowers as large as and larger than those of the *grandiflora* parent and with a similar relation of the stigma lobes to the tips of the anthers. The larger flowers equaled in size those of the largest-flowered types of *Lamarckiana*. Other plants bore flowers approaching in size those of the *biennis* parent, but never so small. There was no fixed relation of the larger flowers to the plants more *grandiflora*-like in foliage or of the smaller flowers to those more *biennis*-like, although larger and smaller flowers were in some instances found on plants approaching the respective parents.

The mass of the culture was fairly close to the F_2 hybrid plant 11.30*Lb*, varying from it in habit, and in the form and measurements of the leaves and flower parts. There was not so strongly marked a tendency on the part of the plants towards the *grandiflora* parent as was exhibited by the former culture. The flowers, as indicated above, showed the same progressive advance in size, many of them being larger than those of *grandiflora* and none so small as those of the *biennis* parent.

A count of the plants made with Mr. Bartlett led to a classification of 104 red-stemmed and 90 green-stemmed in the group of 833 plants. The remainder of the culture presented intermediate or mottled coloration similar to

the F_1 hybrid plant (10.30*Lb*) from which they came. The red-stemmed plants were most numerous towards the more *grandiflora*-like end of the culture and the green-stemmed towards the more *biennis*-like end.

A larger number of remarkable new types appeared in this culture than in the former (11.41), types specifically different from either *biennis* or *grandiflora,* or the F_1 hybrid plant 10.30*Lb*. The list is as follows:

11.42*e,* a type represented by 4 plants with small and narrow leaves, and medium-sized flowers.

11.42*f,* leaves small, irregularly toothed, flowers medium-sized, capsules large (about 3.3 cm. long); represented by several plants and intergrading with the mass of the culture.

11.42*g,* medium-sized plant with large flowers and large crinkled leaves; a type rather common and intergrading with the mass of the culture.

11.42*j,* a single plant with almost linear leaves, flowers very small (petals 6 mm. long), anthers sterile. In its foliage this plant seems very close to De Vries's "mutant" *Œnothera elliptica.*

11.42*l,* medium-sized flowers; remarkable for its broad, entire, and very much crinkled leaves.

11.42*r,* the class separated as 147 small rosettes with narrow leaves of which 90 were saved and grown to maturity. The mature plants, 3–4 dm. high, rarely branched, bore medium-sized flowers, and constituted a clearly defined class of dwarfs.

There are not included in the above list the numerous segregates with characters in various combinations approaching one or the other of the parents of the cross. Some of these types clearly differed specifically from the F_1 hybrid plant 10.30*Lb*.

A consideration of these two cultures (11.41 and 11.42) of hybrids in the second generation will bring out certain important conclusions that may be summarized.

1. In the immensely greater diversity exhibited by the F_2 generation over that of the F_1 is clearly shown a

differentiation of the germ-plasm expressed by the appearance in the F_2 plants of definite tendencies in different directions towards the parents of the cross. This sems to the writer the essential principle of Mendelism and does not necessarily involve the acceptance of the doctrine of unit characters and their segregation in either modified or unmodified form.

2. Certain characters of the parent species have appeared in the F_2 segregates in apparently pure condition, but the very large range of intermediate conditions indicates that factors governing the form and measurements of organs (if present at all) must in some cases be concerned with characters so numerous and so small that they can not be separated from the possible range of fluctuating variations. If this is true such characters seem beyond the possibility of isolation and analysis and the unit character hypothesis for these cases has little more than a theoretical interest.

3. Both cultures certainly showed a marked progressive advance in the range of flower size, the largest flowers having petals somewhat more than 1 cm. longer than those of the *grandiflora* parent. There was a similar advance in the size of the leaves and the extent of their crinkling. These progressive advances would seem to demand on the unit character hypothesis either the modifications of the old or the creation of new factors.

4. The absence of classes among the F_2 hybrids (except for the dwarfs) further works against the unit character hypothesis as of practical value in the analysis of a hybrid generation of this character. It should be remembered, however, that there were in this cross no sharply contrasted distinctions of color, anthocyan coloration proving most unsatisfactory for the purposes of a genetical study.

5. Both cultures presented a class of dwarfs, very different from one another and from the F_1 hybrid plants 10.30*La* and 10.30*Lb*. This phenomenon of nanism has

appeared also in all previous F_2 generations that I have grown and presents a most interesting subject for study.

6. The extreme variants of the cultures would rank as new species different from either parent of the cross and from the F_1 hybrid plants. Their germinal constitution (provided it is stable) on the hypothesis of unit characters apparently must involve the modification of old factors or the creation of whole sets of new factors in large numbers, a degree of complication unfavorable to the hypothesis.

7. The progeny of the F_1 hybrid plant 10.30*La* was very different from that of 10.30*Lb* although these two plants were sisters. What would have been the complications if F_2 generations had been grown from a hundred or a thousand sister plants!

7. The Probable Composition of the Cultures of Carter and Company from Evidence Furnished by the Sheet in the Gray Herbarium

Brief reference was made in a former paper (Davis, '11, p. 228) to a very interesting sheet in the Gray Herbarium of Harvard University, which shows a plant with characters in part those of *Œnothera Lamarckiana,* but, in the writer's opinion more largely those of *grandiflora.* This sheet bears notes in the handwriting of Dr. Asa Gray to the following effect—in ink, "*Œ. Lamarckiana,*" "Hort. Cantab. 1862," and "from seed of Thompson, Ipswich"; in pencil and probably of a different date "said by English horticulturists to come from Texas." It was the habit of Dr. Gray at that time to use herbarium labels marked Hort. Cantab. and this fact, together with the absence of other writing on the sheet indicates that the plant was grown in the botanical garden at Cambridge, Massachusetts.

The date, 1862, suggested a possible genetic relationship of this plant in the Gray Herbarium to the cultures of Messrs. Carter and Company of London about 1860 of plants which were grown for the market under the name

Œnothera Lamarckiana and which seem to have been the starting point of the *Lamarckiana* now under cultivation. The only descriptions which we have of these cultures are the very unsatisfactory accounts in *The Floral Magazine,* Vol. II, Plate 78, 1862, and in *"L'Illustration Horticole,"* Vol. IX, Plate 318, 1862, both accompanied by the same figure of an impossible *Œnothera.*

An inquiry was at once started to determine the meaning of the note "from seed of Thompson, Ipswich," and, thanks to the courtesies of correspondents, the matter now seems clear. There seems to have been no botanist or horticulturist of the name of Thompson in Ipswich, Massachusetts, at that period from whom Dr. Gray could have obtained this seed. The reference is almost certain to have been to William Thompson of Ipswich, England, who died several years ago. William Thompson "was a seedsman in a small way of business, but a most enthusiastic cultivator with correspondents in all temperate countries and the introducer of numerous herbaceous plants more particularly annuals and biennials." He corresponded with Kew as early as 1860.

It is in a high degree probable that William Thompson, with his interest in novelties, obtained from Carter and Company their new *Œnothera* and that the seed sent to Dr. Gray was either directly from this source or from plants cultivated by Thompson. It will be remembered that Carter and Company stated that they received their seed from Texas, which accords with Dr. Gray's note "said by English horticulturists to come from Texas." If this interpretation of the history of the sheet in the Gray Herbarium is correct the plant was very close indeed to the cultures of Carter and Company, possibly not more than one or two generations removed, since these plants were probably cultivated as biennials. This sheet then gives evidence on the composition of the cultures of Carter and Company immensely more valuable than the obviously inaccurate plate of *The Floral Maga-*

zine and the description that tells little of value for the problem under consideration.

The following is a description of this sheet in the Gray Herbarium:

1. *Stems and Foliage.*—The stem bears long hairs arising from papillæ (glands) which are colored red as in *Lamarckiana* and are about as numerous as in that species. A detached leaf (Fig. 15, *a*), about 18.5 cm. long with sinuate margins, slightly lobed below, and with some evidence of former crinkles, suggests by its shape (although too small) the basal leaves of *Lamarckiana.* The upper foliage is similar to a broad-leaved type of *grandiflora,* the leaves being short-petioled and not so nearly sessile as in *Lamarckiana.*

2. *Inflorescence.*—The inflorescence has longer internodes than in *Lamarckiana* and consequently is not so compact; in this respect it resembles *grandiflora.* The bracts are broad at the base, slightly toothed, and persistent, becoming lanceolate leaves on the fruiting branches as in *grandiflora.*

3. *Buds.*—The buds (Fig. 15, *b*) are about 9.5 cm. long, not stout and 4-angled as in *Lamarckiana,* but with a cone circular in section as in *grandiflora.* Sepals apparently green, their tips attenuate as in *grandiflora* and projecting 1 cm. beyond the folded petals; pubescent, with long hairs arising from papillæ among much shorter sessile hairs as in *Lamarckiana.*

4. *Flowers.*—The flowers are somewhat larger than those of any *grandiflora* known to me. Petals about 4.5 cm. long, as long as those of the largest forms of *Lamarckiana.* Stigma lobes about 8 mm. long, close to 5 mm. above the tips of the anthers, in these respects agreeing with both *Lamarckiana* and *grandiflora.*

5. *Capsules.*—The capsules, about 3 cm. long, are of medium thickness and similar to those of *grandiflora;* they are not so stout as the capsules of *Lamarckiana.* The persistent leafy bracts and long internodes give the

fruiting branches a marked resemblance to the conditions characteristic of *grandiflora.*

This plant then presents *grandiflora* characters in the upper foliage of the plant, in the longer internodes of the

FIG. 15. Sheet in the Gray Herbarium of Harvard University. An *Œnothera* grown by Dr. Asa Gray at Cambridge, Massachusetts, in 1862 and probably derived directly or indirectly from the cultures of Carter and Company of London, which were distributed under the name *Lamarckiana.* The specimen on this herbarium sheet has important characters of *grandiflora,* indicating a relationship to this species.

inflorescence and the persistent bracts, in the form of the buds (4-angled) with attenuate sepal tips, and in the longer capsules of medium thickness. The plant resembles *Lamarckiana* in the red coloration of the papillæ on

the stem at the base of the long hairs, and in the form and size of a large detached leaf (probably basal). The form of the flowers is essentially similar to either *grandiflora* or the large-flowered types of *Lamarckiana;* their size is very close to that of the latter plant, which it also resembles in the pubescence of the sepals. In this mixture of characters the most important are, in the writer's opinion, distinctly *grandiflora*-like and indicate a close relationship to some strain of *grandiflora.*

The short description in *The Floral Magazine,* Vol. II, 1862, quotes the following from Carter and Company. "We received, about four years ago, some seed from Texas unnamed. When we had flowered it, we sent some blooms to Dr. Lindley, who pronounced it to be *Œnothera Lamarckiana,* a species, we believe, introduced into England by Mr. Drummond. Its height is between three and four feet; it blooms the first year, is a very hardy biennial, and is superior to any other *Œnothera* in the size and number of its blossoms, which measure four inches in diameter." Of the characteristics noted in this quotation, all of which fit *Lamarckiana,* the most important is the statement that the plant is "a very hardy biennial," although this is somewhat weakened by the remark that " it blooms the first year."

Now *grandiflora* is clearly annual and the *Œnotheras* of the south and southwestern United States are, as far as the writer is aware, generally annual or perennial. A well-defined biennial habit, characterized by the development of large and short persistent rosettes, is an adaptation to the short seasons of northerly climates. The question arises whether the plants raised by Carter and Company are represented or could have been represented in the Texan flora. We know that Texas and the southwest generally have some large-flowered *Œnotheras* and it may be that the climatic conditions of certain mountainous regions would favor the development of a biennial habit. However, botanical exploration has not yet brought forward any plant of the *Lamarckiana* type. In the absence of

corroborative evidence we can hardly at present accept as beyond doubt the statement that the seeds of Carter and Company were from Texan plants.

It is a confused situation with a number of possible explanations which are not worth a discussion until we have more evidence at hand. This evidence may come through other herbaria sheets comparable to the one in the Gray Herbarium, although examinations by my correspondents of the collections at the British Museum, at Kew, and at Cambridge University indicate that there is nothing at these centers. Evidence may also come from field studies in America, which should be pushed by western and southern botanists in a position to observe *Œnothera* throughout the season. The writer sowed about 200 seeds (60 years old) from one capsule on the sheet in the Gray Herbarium, but there have been no germinations after being four months in the seed pan; the experiment, a forlorn hope, needs no apology, considering the importance of the problem—the composition of the cultures of Carter and Company.

The most important point for the writer's hypothesis of the hybrid origin of *Œnothera Lamarckiana* as a cross between *biennis* and *grandiflora* is the strong evidence that the cultures of Carter and Company contained forms with characters in part *grandiflora*-like and in part *biennis*-like, for it must be remembered that *Lamarckiana* in its hardy biennial habit and in other characters resembles the latter species. It is possible that the plants were hybrids at that period (1860) for if the sheet in the Gray Herbarium is representative of the composition of these cultures, the plants were very different from the present day *Lamarckiana*. Even if the plants of Carter and Company came from Texas as a form related to *grandiflora* there would of course have been abundant opportunities for hybridization with *biennis* before De Vries, a quarter of a century later, began his studies.

8. Further Considerations on the Possible Origin of *Œnothera Lamarckiana* as a Hybrid of *O. biennis* and *O. grandiflora*

Among the most interesting of the results from the cultures of the past summer (1911) have been those concerned with the behavior of the hybrids of *biennis* and *grandiflora* in the second generation. In the progeny of the F_1 hybrid plants 10.30*La* and 10.30*Lb* there was clear evidence of an advance in the size of the flowers which carried them in many plants beyond the size of the *grandiflora* parent and as far as the larger-flowered forms of *Lamarckiana*. The petals of these F_1 hybrid plants measured about 2.2 cm.; those of the largest of the F_2 flowers measured 4.5 cm.; *grandiflora B* has petals about 3.3 cm. long and those of the largest-flowered forms of *Lamarckiana* measure about 4.5 cm. There was a similar advance in the size of the leaf and the extent of its crinkling, although the leaves did not reach the conditions characteristic of *Lamarckiana*. This is clearly progressive evolution and doubtless can be maintained through selection, though there may be in later generations constant retrogressive variation.

This demonstration of a progressive advance in flower measurements in the F_2 generation disarms those critics (as Gates, '11) who in prematurely discussing my F_1 hybrid plants 10.30*La* and 10.30*Lb* apparently failed to appreciate the basic principle that the characteristics of an F_1 hybrid are shown by what it will do in the F_2 generation and not by what it may seem to be. No experimenter in touch with present-day genetics would hope to get a perfect *Lamarckiana* type in the F_1 generation from a cross between *biennis* and *grandiflora*, for the reason that the characters of these species could neither blend nor appear as dominant or recessive in such a manner as to give this type. But with a proper selection of parent biotypes there is good reason to be hopeful that from the F_2 generation of such a cross forms will appear with

characters so shifted and modified as to match very closely those of *Lamarckiana*.

My hypothesis does not of course demand that *Lamarckiana* arose with all of its characters fully present as the result of a simple cross. There have been abundant opportunities for repeated crosses of a most varied character during the long period in which this plant has been under cultivation. That the plant hybridizes readily in nature is evidenced by the complex assemblage of varied types found in such localities as the sandhills of Lancashire, England, where *Lamarckiana*-like forms are growing wild in great numbers together with types of *biennis*. The fundamental feature of the hypothesis is the belief that *Lamarckiana* exhibits the behavior to be expected of a complex hybrid and that its characteristics are those likely to come from a mixture of germ-plasms from types of *biennis* and *grandiflora*.

I shall not, in order to perfect a synthesis of *Lamarckiana*-like hybrids, depart from my plan of experimenting with wild races of *biennis* and *grandiflora*, for the most important feature of the line of experimentation under way is the study of the behavior of species hybrids. Such a study offers the opportunity of treating experimentally one of the most vital problems of genetics,—the possibilities and methods of the progressive evolution of specific characters through hybridization.

The studies have not yet proceeded sufficiently far to justify a detailed comparison between the various forms of F_2 hybrids and the "mutants" of De Vries. It is clear, however, that the F_2 generation will give numbers of types that differ from F_1 hybrid plants *in the same manner* as the "mutants" differ from *Lamarckiana*. Some of the variants have characters so different from the F_1 parent hybrid plants that they would stand in taxonomic practise as new species, others have differences of such a nature that they might be called progressive, or retrogressive varieties. The chances are immensely against obtaining a *Lamarckiana*-like hybrid

which will produce the same series of variants as the "mutants" obtained by De Vries, for the reason that no two F_1 hybrid plants in so complex a cross as that between *biennis* and *grandiflora* are likely to give exactly the same set of variants. This principle is strongly indicated in the diverse F_2 progeny of the two sister plants 10.30*La* and 10.30*Lb*.

It is, naturally, important for my hypothesis that *Lamarckiana*-like hybrids continue to give in successive generations similar variants after the manner of *Lamarckiana,* but, as noted above, it is not necessary that they be taxonomically the same forms (*i. e., gigas, rubrinervis, nanella,* etc.), or that they be produced in the same proportions. Upon these points we shall sooner or later have definite data.

The results from the work of the past summer (1911), described in this paper, have strengthened the writer's hypothesis in the following respects:

1. A definite advance has been made in obtaining more favorable F_1 hybrids by employing a biotype of *biennis* (*biennis D*) with characters closer to those of *Lamarckiana* than the characters of *biennis A* and *B* previously used.

2. The F_2 generations from the hybrid plants 10.30*La* and 10.30*Lb* have shown that large numbers of variants may arise from a cross between *biennis* and *grandiflora,* some of apparently new specific rank, others exhibiting variations of either a progressive or retrogressive nature, and very many presenting in various degrees of complexity a segregation of the characters of the parents.

3. The progressive advance in flower and leaf size over that of the parents of the cross indicates that selection of the sort common among gardeners (*i. e.,* the choice of the largest and most vigorous plants) might readily establish a race surpassing in these and other respects both parent plants. It is exactly this sort of selection which, in the writer's opinion, has established the larger-flowered forms of *Lamarckiana.*

It is to be regretted that the terms mutant and mutation are being used so variously by different workers in the fields of genetics. "Mutations" have been described which are obviously from heterozygous parentage, in some cases small differences, as of color or measurement, in other cases very large differences. In contrast to these have been described "mutations" from possible homozygous parentage. Germinal variation due to the mixing of different germ-plasms, and consequently from heterozygous material, is a common phenomenon and easily defined. Germinal variation in homozygous material presents an equally clear concept. The two types of variation should be sharply distinguished for one of the most important fields of genetical research is the study of possible germinal variations in homozygous stock and the conditions under which they may occur.

In our understanding of germinal variations (mutations) as distinguished from somatic variations (fluctuations) a great advance has been made towards a clear appreciation of the problems involved. The problems center in the study of germinal variations which are of two types since they may be due to the mingling of germ plasms (amphimixis), or to environmental influences. Amphimixis is of course responsible for heterozygous conditions now better understood as a result of Mendelian studies. Relatively little is known, however, of the conceivable effects of environment as a source of germinal variation. It may be doubted whether the specific terms "mutation" and its alternative "fluctuation," as commonly used, are well chosen since the concepts are so clearly expressed by the designations germinal and somatic variations.

It is clear that De Vries regarded the "mutants" of *Lamarckiana* as variants from a type representative of a wild species and as nearly homozygous as most well-defined species. That the *Lamarckiana* with which De Vries worked was strongly heterozygous, in fact a hybrid of *biennis* and *grandiflora*, is the hypothesis for which I am trying to present as much evidence as possible. By

this hypothesis, if finally accepted, most of the "mutants" of De Vries are likely to fall into the class of variants due to the mixing of different germ-plasms. If the word mutation, in the sense of De Vries, is to have a meaning more precise than that of variant it must be kept for the type of variation from homozygous stock.

That germinal variations may occur in homozygous material seems to the writer more than probable, and it is possible that many *small* variations are of this type. The trend of experimental investigation, however, distinctly indicates that large variations (ranking as saltations) are rare if present at all in homozygous material, and consequently can not be important factors in organic evolution. The variations considered by Darwin are chiefly either the small variations in relatively homozygous forms, or the large and small variations from heterozygous stock.

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